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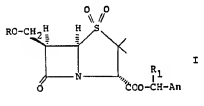
(54) **B-lactam antibiotics**

(57) The present invention relates to hitherto unknown β-lactam compounds including their salts with pharmaceutically acceptable, non-toxic acids or bases, to methods for producing said new compounds, to dosage units of the compositions, and to methods of treating patients suffering from infectious diseases using the said new compounds and compositions.

The compounds of the invention are represented by the general formula I:



I

in which R = H, C_1-C_4 alkyl or C_2-C_5 acyl, R_1 stands for hydrogen, methyl, ethyl, benzyl, or phenyl, and An stands for a radical of a cephalosporin-type or an amidinopenicillanic acid being connected via the carboxy group.

The compounds of the invention, which are valuable antibiotics in the human and veterinary therapy, are useful in the treatment of bacterial infections. They are in particular strongly active against β-lactamase producing bacteria.

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The Chemical formula(e) appearing in the printed specification were submitted afer the date of filing, the formula(e) originally submitted being incapable of being satisfactorily reproduced.

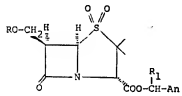
SPECIFICATION

β-Lactam antibiotics

The present invention relates to hitherto unknown β-lactam compounds including their salts with pharmaceutically acceptable, non-toxic acids or bases, to methods for producing said new compounds, to dosage units of the compositions, and to methods of treating patients suffering from infectious diseases using the said new compounds and compositions.

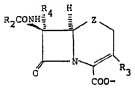
The present invention provides new compounds useful in the treatment of bacterial infections. The new compounds are in particular strongly active against β-lactamase producing bacteria.

The compounds of the invention, which are valuable antibiotics in the human and veterinary therapy, are represented by the general formula I:



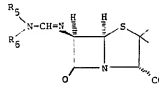
I

in which R = H, C_1-C_4 alkyl or C_2-C_5 acyl, R_1 stands for hydrogen, methyl, ethyl, benzyl, or phenyl, and An stands for a radical of a cephalosporin-type or an amidinopenicillanic acid being connected via the carboxy group. More specifically, An is the acyloxy radical represented by one of the general formulae II or III:



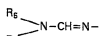
IIa, Z = S

IIb, Z = O



III

in which formulae R_2CONH- stands for the 7-side chain of a known cephalosporin, R_3 stands for the 3-substituent of a known cephalosporin, R_4 stands for hydrogen or methoxy, and



stands for the side chain of a known amidinopenicillanic acid.

Useful cephalosporins forming radicals of formula IIa are for instance described in: E.H. Flynn, Cephalosporins and Penicillins, Academic Press, New York and London 1972, P.G. Sammes, Chem. Rev., 1976, Vol. 76, No. 1, pages 113-155, J. Cs. Jásberényi and T.E. Gunda, Prog. Med. Chem., Vol. 12, 1975, pages 395-477, J. Elks, Recent Advances in the Chemistry of β-lactam antibiotics, The Chemical Society, London 1977, Merck Index, 9. Edition, Merck and Co., Inc., Rahway, N.J., USA, 1976, and Encyclopedia of Antibiotics, 2. Edition, John Wiley and Sons (1978), Chemistry and Biology of β-Lactam Antibiotics, Vol. 3, Academic Press, New York and London 1982, pages 387-392.

Useful amidinopenicillanic acids forming radicals of formula III are for instance those described in British patents Nos. 1,293,590 and 1,315,566, and in German patent No. 2,055,531.

Of particularly useful cephalosporins forming radicals of formula IIa, mention may be made of cefoxitin, cefuroxime, cefotaxime, ceftizoxime, cefamandole, cefsulodin, cefotiam, cefmenoxime, cefoperazone, cefmetazole, cephaloglycin, cephalexin, cephradine, cefadroxil, cefaclor, cefatrizine, cephaloridine, cephapirin, cefazolin, ceftezole, cefazetrile, ceftazidime, and cefroxadrine.

A particularly useful 1-oxacephalosporin of formula IIb is moxalactam.

Of particularly useful amidinopenicillanic acids forming radicals of formula III, mention may be made of mecillinam.

The above mentioned Examples of particularly useful cephalosporins and amidinopenicillanic acids shall only be considered illustrating and not limiting the scope of the present invention.

Of particular interest are compounds of formula I, in which R_1 and R both represent hydrogen, in particular such compounds in which An is a radical formed by cephradine, cefaclor, cephalexin or mecillinam.

The present invention covers all possible diastereomeric forms of the compounds of formula I as well as mixtures thereof.

As stated above, the invention also relates to salts of the esters of formula I with pharmaceutically acceptable, non-toxic acids or bases, depending upon whether An contains a basic or acidic function.

Among suitable acids can be mentioned hydrochloric acid, hydrobromic acid, hydroiodic acid, phosphoric acid, sulphuric acid, nitric acid, p-toluenesulphonic acid, methanesulphonic acid, 2-naphthalenesulphonic acid, formic acid, acetic acid, propionic acid, citric acid, tartaric acid, maleic acid, pamoic acid, and p(dipropylsulfamyl)benzoic acid (probenecid). Among suitable bases can be mentioned alkali metal or alkaline earth metal hydroxides, such as sodium, potassium, magnesium, or calcium hydroxide, as well as ammonia or suitable non-toxic amines, such as lower alkylamines, e.g. triethylamine, hydroxy-lower alkylamines, e.g. 2-hydroxyethylamine, bis-(2-hydroxyethyl)-amine or tris-(2-hydroxyethyl)-amine, cycloalkylamines, e.g. dicyclohexylamine, or benzylamines, e.g. N,N′-dibenzylethylenediamine or dibenzylamine, without these examples being limiting the invention. Also salts which acidic or basic antibiotics are within the scope of the invention. In some instances, it is preferred to use easily soluble salts, whereas for other purposes, it may be appropriate to use an only slightly soluble salt, such as the napsylate or tosylate salts, e.g. in order to obtain a prolonged effect. In particular, a prolonged effect can be obtained by using a salt with probenecid which blocks the tubular excretion of β-lactam compounds.

In clinical treatment of bacterial infections it is a serious problem that β-lactamase producing bacteria are occurring with increasing frequency. These enzymes are capable of inactivating β-lactam antibiotics, and it is well recognized that β-lactamases from both gram-positive and gram-negative bacteria contribute significantly to the resistance of bacteria to β-lactam antibiotics.

Several naturally occurring β-lactamase inhibitors, including clavulanic acid and the olivanic acids, have been described, and also a number of semisynthetic β-lactam compounds, e.g. penicillanic acid 1,1-dioxide, 6α-chloropenicillanic acid 1,1-dioxide, a series of clavulanic acid derivatives, 6β-halopenicillanic acids, such as 6β-iodo- and 6β-bromopenicillanic acid, methicillin sulphone, and quinacillin sulphone, were found to possess similar biological properties. With a few exceptions, these compounds display only weak antibacterial activity against most gram-positive and gram-negative organisms, but are powerful inhibitors of a variety of β-lactamases.

A particularly useful β-lactamase inhibitor is 6β-hydroxymethylpenicillanic acid sulphone, the preparation of which has been described in Belgium patent No. 885,812 and in Swiss patent application No. 4017/80. We have found that it is a potent inhibitor of various types of β-lactamases, including cephalosporinases, against which other known inhibitors are only weakly active.

In order to combat a bacterial infection successfully, it is known to use a combined treatment with both one or more β-lactam antibiotics and one or more β-lactamase inhibitors.

According to the present invention, such combined treatment can with advantage be undertaken with a compound of formula I which after absorption and cleavage in the body is converted into two active drugs, a β-lactam antibiotic and a β-lactamase inhibitor. The compounds of formula I can thus be considered mutual prodrugs for the antiobiotic and the inhibitor in question. However, two prerequisites are necessary to utilize this feature of the new compounds of formula I. They must be capable of being absorbed from the gastro-intestinal tract, and during or after absorption they must be hydrolyzed with liberation of the antibiotic and the inhibitor in question. It has turned out that both of these prerequisites are fulfilled, and therefore the present compounds are valuable and active compounds for their intended purposes.

Thus, studies in animals have shown that the new compounds are readily absorbed from the gastro-intestinal tract. During or after absorption they are hydrolyzed with liberation of equimolar amounts of the two components in question, giving rise to simultaneous high blood and tissue levels of the two components. Thereby the antibiotic is in the most effective manner protected by the inhibitor against inactivation.

By using the compounds of the invention the antibacterial spectrum of the β-lactam antibiotic in question is widely extended, as also β-lactamase producing strains will be susceptible to treatment. Such β-lactamase producing strains are found with increasing frequency and are as mentioned above a serious problem in clinical therapy. The compounds of the invention will for such purpose be of extreme value.

Therapeutically, the new compounds have distinct advantages over more combinations of the β-lactam antibiotic and the β-lactamase inhibitor to which they are hydrolyzed, or combinations of orally active esters thereof.

For example, many β-lactamase inhibitors, including 6β-hydroxymethylpenicillanic acid 1,1-dioxide, are absorbed incompletely or irregularly from the gastro-intestinal tract. Also many of the antibiotics forming part of the present compounds are incompletely absorbed, when given orally. In addition, individual variations in the rate of absorption of the various antibiotics and the β-lactamase inhibitor may in many instances lead to a situation where the active components will not be present simultaneously or in the

optimum ratio at the site of infection, even if the two drugs were given at the same time.

Certain easily hydrolyzable esters of the antibiotics and the β-lactamase inhibitors, the radicals of which are linked in the compounds of formula I, are absorbed better from the gastro-intestinal tract than the corresponding free acids. However, hydrolysis of such esters in the organism gives rise to the formation of inactive by-products, and although these by-products are relatively non-toxic, it is undesirable to expose the organism to unnecessary metabolites. Another disadvantage of using combinations of easily hydrolyzable esters of the antibiotic and the β-lactamase inhibitor is that the ester moieties increase the molecular weight of the compounds and consequently the size of the dosage unit. By using the compounds of the invention, the size of the dosage units can be decreased considerably.

In addition, the absorption of such esters will normally not take place simultaneously, even if the compounds are given to the patient at the same time.

All of these disadvantages are avoided by using the compounds of the invention.

It has been found that *in vitro* synergy between 6β-hydroxymethylpenicillanic acid-1,1-dioxide and various cephalosporins and substituted amidinopenicillanic acids is particularly pronounced when the ratio between the two components is between 3:1 and 1:3. As the various antibiotics have different biological half-lives and distribution characteristics, the ratio between the liberated components of the new compounds in the organs and tissues may vary to some degree, but will normally be within the above preferred limits.

The synergistic effect are illustrated in the Table:

TABLE

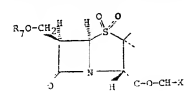
Susceptibility [x] of β-lactamase-producing bacterial strains to 6β-hydroxymethyl-1,1-dioxopenicillanic acid (A), to three cephalosporins, and to compounds of the invention containing moities of cephalosporins and A, as indicated. The compounds were subjected to enzymatic cleavage prior to testing

	Test Organism		
Test Compound	*Enterobacter Cloacae HC4*	*Enterobacter Cloacae P99 HC8*	*Proteus Vulgaris HJ33*
A	20	50	>100
Cephalexin	80	>300	>300
Cephalexin + A	5 + 5	16 + 16	16 + 16
Cefaclor	12	300	ND [xx]
Cefaclor + A	0.8 + 0.8	6.3 + 6.3	ND [xx]
Cephradine	40	>300	>300
Cephradine + A	5 + 5	10 + 10	16 + 16

[x] Expressed as IC_{50}-values in μg/ml, determined on agar medium according to *Antimicrobial Agents and Chemotherapy, 8*, 266 and 271 (1975).

[xx] Not determined

The present invention also includes methods for the preparation of the new compounds and their salts. According to one method a compound of formula IV:



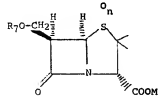
IV

in which R_1 is as defined before, R_7 is optionally R (as defined above) or an easily cleavable esterifying or etherifying group, such as benzyloxycarbonyl, 2,2,2-trichloroethoxycarbonyl, benzyl, 2,2,2-trichloroethyl, or trialkylsilyl, e.g. tert-butyldimethylsilyl, and X stands for a leaving group, such as a halogen atom, preferably iodine, is reacted with a compound of formula $An^{*\ominus}M^{\oplus}$, in which $An^{*\ominus}$ is the carboxylate ion corresponding optionally to An (as defined above) or to a protected or masked derivative of An, e.g. containing an azido group or a benzyloxycarbonyl group instead of an amino group present in An, or An^* is an appropriate penicillin-type radical which can be rearranged at a suitable stage to a cephalosporin-type radical An, and $M^{\oplus}$ is a cation, such as Na^+, K^+, an ammonium ion, a tri- or tetraalkylammonium ion, e.g.

tetrabutylammonium ion, followed, when $R_7 \neq$ and/or An* $\neq$ An, by appropriate chemical modifications, e.g. cleavage to replace R_7 with hydrogen and then optionally alkylation or acylation, and/or reactions required to convert An* to An, e.g. hydrogenation of an azido group or a benzyloxycarbonyl group to an amino group, to give the desired compound of formula I.

The reaction between IV and $An^{*\ominus}M^{\oplus}$ is performed in a suitable solvent, e.g. dimethylformamide, ethyl acetate, dichloromethane, acetone or hexamethyl phosphoric acid triamide, for a sufficient time and at an adequate temperature with a view to accomplish the desired conversion, usually at a temperature from 0°C to 60°C.

Compounds of formula IV are prepared by reacting a compound of formula Va



Va, n = 2
Vb, n = 0

with a compound of formula VI

VI

in which R_1 and X are as defined before, and Y represents a leaving group, such as bromine or iodine, halosulphonyloxy, e.g. chlorosulphonyloxy, alkylsulphonyloxy, α-haloalkoxysulphonyloxy, or unsubstituted or substituted arylsulphonyloxy, such as benzenesulphonyloxy, tosyloxy, or bromobenzenesulphonyloxy, Y being a better leaving group than X, followed by substituting X in formula IV for a better leaving group, if desired.

Alternatively, compounds of formula IV may be prepared by reacting a compound of formula Vb with a compound of formula VI, followed by oxidation. Again substitution of X in formula IV for a better leaving group may be peformed, if desired.

The oxidation step may be performed for example by using a peracid, e.g. m-chloroperbenzoic acid, in an inert solvent, e.g. CH_2Cl_2, or by using potassium permanganate in a solvent mixture containing acetic acid.

The compounds of formula I can also be prepared according to a method in which as a first step a compound of formula $An^{*\ominus}M^{\oplus}$ is reacted with a compound of formula VI to afford an intermediate of formula VII:

VII

in which A*, R_1 and X have the above meanings.

The reactions between VI and V or $An^{*\ominus}M^{\oplus}$ are performed in a suitable solvent, e.g. dimethylformamide, ethyl acetate, dichloromethane, acetone or hexamethyl phosphoric acid triamide, usually at a temperature from 0°C to 60°C.

A preferred compound of formula VI is chloromethyl chlorosulphate, and the preferred reaction conditions involve the use of a two-phase solvent system of an organic solvent and water and a phase transfer catalyst, such as tetrabutylammonium hydrogen, sulphate, in the presence of a base, e.g. $NaHCO_3$.

In a second step the intermediate of formula VII is reacted with a compound of the formula Va to form the desired compound of formula I, if necessary after appropriate modification of R_7 to R and for An* to An.

If desired, X in formula VII can in advance be exchanged for a better leaving group, and modification of An* (for example oxidation and ring expansion of a penicillin-type An* to a cephalosporin-type An*) may be performed at the formula VII stage.

The reaction between VII and Va is performed in a reaction-inert organic solvent, e.g. dimethylformamide, ethyl acetate, dichloromethane, acetone or hexamethyl phosphoric acid triamide under conditions mentioned above, and usually at temperatures between 0°C and 60°C.

This reaction is followed by the necessary modification of functionality required to convert An* to An and/or R_7 to R, if required.

Some of the intermediates of formula IV, Va, Vb, and VII are hitherto unknown compounds, and thus form part of the present invention.

The starting materials of formula VI are known or may be prepared by methods analogous to those used for the preparation of similar known compounds.

The compounds of formula I can be purified and isolated in usual manner and may be obtained either as such or in the form of a salt.

The compounds may in some cases be obtained as diastereomeric mixtures which, when desired, may be separated by known methods e.g. chromatography.

It is a further object of the present invention to provide pharmaceutical compositions which are useful in the treatment of infectious diseases in the human and veterinary practice, and which may be used for enteral, parenteral or topical administration.

With this object in view, the compositions of the invention contain as an active component at least one member selected from the group consisting of compounds of the formula I and salts thereof as defined above, together with solid or liquid pharmaceutical carriers and/or diluents.

In the said composition, the proportion of therapeutically active material to carrier-substance can vary between 1% and 95% by weight. The compositions can be worked up to various pharmaceutical forms of presentation, such as tablets, pills, dragees, suppositories, capsules, sustained-release tablets, suspensions and the like containing the compounds of formula I or their atoxic salts, mixed with carriers and/or diluents.

Pharmaceutically acceptable, non-toxic, organic or inorganic, solid or liquid carriers and/or diluents can be used to make up compositions containing the present compounds. Gelatine, lactose, starch, magnesium stearate, talc, vegetable and animal fats and oils, gum, polyalkylene glycol, buffers or other known carriers, auxiliary agents and/or diluents for medicaments are all suitable.

Furthermore, the compositions may contain other therapeutically active components which can appropriately be administered together with the present compounds in the treatment of infectious diseases, such as other antibacterials, antitussiva, pain-relieving drugs, probenecid, etc. In particular, antibacterials, such as penicillins or cephalosporins, which act synergistically with one or both of the active components formed by *in vivo* hydrolysis of the compounds of the invention, are appropriate. The ratio between the therapeutically active components, e.g. the present compounds together with a known penicillin, can vary between 1:20 and 20:1, preferably between 1:5 and 5:1.

A particularly advantageous compounded composition contains as active ingredients 6β-hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 6β[(hexahydro-1H-azepin-1-yl)-methyleneamino]penicillanate, optionally being used in the form of a salt with a pharmaceutically acceptable, non-toxic acid and 6-[D-α-amino-α-phenylacetamido]penicillanic acid.

The compounds of formula I can be used either as such or in the form of a salt. The compounds as such are only slightly soluble in water, whereas many of the salts, e.g. the hydrochlorides when An contains an amino group, are readily soluble in water.

As indicated above, the present compounds may be worked up to pharmaceutical forms of presentation including suspensions and non-aqueous ointments. A pharmaceutical preparation for oral treatment may be in the form of a suspension of one of the present compounds, the preparation containing from 10 mg to 100 mg per ml of the vehicle.

Another object of the invention resides in the selection of a dose of the compounds of the invention and a dosage unit of the compositions of the invention which dose and dosage unit can be administered so that the desired activity is achieved without simultaneous secondary effects. In the human therapy, the present compounds are conveniently administered (to adults) in dosage units of the compositions containing not less than 50 mg and up to 2500 mg, preferably from 100 mg to 1000 mg calculated as the compound of formula I.

By the term "dosage unit" is meant a unitary, i.e. a single dose which is capable of being administered to a patient, and which may be readily handled and packed, remaining as a physically stable unit dose comprising either the active material as such or a mixture of it with solid or liquid pharmaceutical diluents, carriers, solvents and/or auxiliary agents.

In the form of a dosage unit, the compound may be administered once or more times a day at appropriate intervals, always depending, however, on the condition of the patient, and in accordance with the prescription made by the medical practitioner.

Thus a daily dose will preferably be an amount of from 0.25 g to 15 g of a compound of formula I or an equivalent amount of a salt thereof as defined before, which conveniently can be divided into several single doses.

In the continuous therapy of patients suffering from infectious diseases, the tablets or capsules are the appropriate form of pharmaceutical preparation, if desired in the form of sustained-release formulations.

In the veterinary practice the above pharmaceutical compositions may also be used, preferably in the form of dosage units containing from 50 mg up to 25 mg of the compound of formula I or a corresponding amount of a salt thereof.

For the treatment of mammary disorders, especially bovine mastitis, the antibacterial agent can be administered by the intramammary route in liquid or semi-liquid form, such as an ointment, or together with a substantially water-insoluble and oil-insoluble binding agent in the form of granules.

Still another object of the invention is to provide a method of treating patients from infectious diseases, the method comprising administering to adult patients an effective amount of a compound of formula I, either as such or in the form of a salt as defined before, and preferably, in the form of the dosage units

aforesaid. The compounds of formula I are typically administered in amounts of 3 - 200 mg/kg body weight of the patient/day, corresponding to, for adult human patients, from 0.25 g to 15 g per day, or an equivalent amount of a salt as defined before of a compound of formula I.

In the treatment of patients, the present compounds can be administered either alone or together with other therapeutically active compounds, e.g. probenecid, which aid in combatting the bacterial infection. Such combined treatment can be performed with formulations containing more or all of the therapeutically active compounds, or these may be administered in separate formulations, these being given simultaneously or with suitable intervals.

In the treatment of patients, the daily dose is administered either at one time, or in divided dosages, e.g. two, three, or four times a day.

The invention will be further described in the following Examples, which are not to be construed as limiting the invention.

With reference to the characterisation of compounds described in the following, it should be noted that melting points (m.p.) were determined on a Büchi-Tottoli apparatus and are uncorrected, and NMR spectra were recorded at 100 MHz on a JEOL FX 100 spectrometer, using $CDCl_3$ as solvent unless otherwise stated. Chemical shifts (δ) are quoted in ppm relative to tetramethylsilane ($\delta = 0$) for $CDCl_3$ solutions or HDO ($\delta = 4.66$) for D_2O solutions. Organic solutions were dried over anhydrous sodium sulphate.

EXAMPLE 1

Chloromethyl 6β-hydroxymethylpenicillanate 1,1-dioxide

To a stirred mixture of 6β-hydroxymethyl penicillanic acid 1,1-dioxide (0.20 g), sodium hydrogen carbonate (0.40 g), and tetrabutylammonium hydrogen sulphate (0.05 g) in dichloromethane (5 ml) and water (2 ml) was added redistilled chloromethyl chlorosulphate (0.10 ml), and stirring was continued at room temperature for 1/2 h. The organic layer was then separated, and the aqueous layer extracted with dichloromethane (10 ml). The organic layers were combined, washed with brine and dried. Concentration *in vacuo* gave a gum which was purified by flash chromatography on silica gel (15 g) using an 80% solution of ethyl acetate in cyclohexane as eluant to give the title compound.
NMR: δ = 1.47 (3H, s), 1.62 (3H, s), 2,49 (1H, bs), 4.2 (3H, m), 4.51 (1H, s), 4.73 (1H, d, J = 4 Hz), 5.65 (1H, d, J = 6.2 Hz), and 5.95 (1H, d, J = 6.2 Hz).

EXAMPLE 2

Iodomethyl 6β-hydroxymethylpenicillanate 1,1-dioxide

Sodium iodide (0.3 g) was added to a stirred solution of chloromethyl 6β-hydroxymethylpenicillanate 1,1-dioxide (0.2 g) in acetone (5 ml), and stirring was continued at room temperature for 2 days. The reaction mixture was partitioned between ethyl acetate (40 ml) and water (40 ml), and the ethyl acetate layer was separated, washed with 5% sodium bicarbonate solution containing a few drops of 10% sodium thiosulphate solution, brine, and dried. Removal of the solvent *in vacuo* gave the title compound.
NMR: δ = 1.49 (3H, s), 1.61 (3H, s), 2.4 (1H, bs), 4.2 (3H, m), 4.45 (1H, s), 4.72 (1H, d, J = 4.5 Hz), 5.89 (1H, d, J = 5.1 Hz), and 6.11 (1H, d, J = 5.1 Hz).

EXAMPLE 3

Chloromethyl 7β-(D-α-azido-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate

A solution of *m*-chloroperbenzoic acid (90%, 10.0 g) in dichloromethane (100 ml) was added dropwise over 2.5 h to an ice-cooled and stirred solution of chloromethyl 6β-(D-α-azido-α-phenylacetamido)penicillanate (22.0 g) in dichloromethane (200 ml), until TLC analysis of the reaction mixture showed complete consumption of the penicillanate starting material. At this point, the mixture was filtered and the filtrate washed with 5% sodium hydrogen carbonate solution (3 × 100 ml), brine, and dried. Removal of the solvent *in vacuo* gave chloromethyl 6β-(D-α-azido-α-phenylacetamido)penicillanate 1-oxide.
NMR: δ = 1.27 (3H, s), 1.74 (3H, s), 4.70 (1H, s), 5.07 (1H, d, J = 4.7 Hz), 5.11 (1H, s), 5.64 (1H, d, J = 6 Hz), 5.94 (1H, d, J = 6 Hz), 5.99 (1H, dd, J = 4.7 Hz and 10 Hz), 7.39 (5H, s), and 8.03 (1H, d, J = 10 Hz).

A solution of the above 1-oxide (10.2 g) and monopyridinium dichloromethanephosphonate (0.25 g) in dry dioxan (70 ml) was heated under reflux under an atmosphere of nitrogen for 3 h. The reaction mixture was cooled and partially concentrated *in vacuo*. The residue was taken up in ethyl acetate (150 ml) and washed with 5% sodium hydrogen carbonate solution (50 ml), brine, and dried. Removal of the solvent gave a foam which was purified by flash chromatography on silica gel (300 g) using a 50% solution of ethyl acetate in petrol as eluant to give a solid which was recrystallised from ethyl acetate - cyclohexane to give the title compound, mp. 134-135°C.
NMR: δ = 2.19 (3H, s), 3.38 (2H, ABq, J = 18 Hz), 4.98 (1H, d, J = 4.7 Hz), 5.10 (1H, s), 5.73 (1H, dd, J = 4.7 and 9 Hz), 5.79 (2H, ABq, J = 6 Hz), 7.25 (1H, d, J = 9 Hz), and 7.39 (5H, s).

EXAMPLE 4

Iodomethyl 7β-(D-α-azido-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate

Sodium iodide (3.5 g) was added to a stirred solution of chloromethyl 7β-(D-α-azido-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate (4.6 g) in acetone (50 ml) and stirring was continued at 40°C for 18 hours. The reaction mixture was partially concentrated *in vacuo* and the residue partitioned between ethyl acetate

(200 ml) and water (200 ml). The organic layer was separated, washed with a 1% solution of sodium thiosulphate (50 ml), water, brine, and dried. Removal of the solvent *in vacuo* gave a solid which was recrystallized from ethyl acetate-cyclohexane to give the title compound as needles, mp. 153-154°C (decomp.).

NMR: δ = 2.19 (3H, s), 3.37 (2H, ABq, J = 18 Hz), 4.97 (1H, d, J = 4.7 Hz), 5.10 (1H, s), 5.73 (1H, dd, J = 4.7 and 9 Hz), 5.98 (2H, ABq, J = 5 Hz), 7.25 (1H, d, J = 9 Hz), and 7.39 (5H, s).

EXAMPLE 5

6β-Hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 7β-(D-α-azido-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate

A solution of iodomethyl 7β-(D-α-azido-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate (150 mg) in dry *N,N*-dimethylformamide (DMF) (3 ml) was added to a stirred suspension of potassium 6β-hydroxymethylpenicillanate 1,1-dioxide (75 mg) in dry DMF (1 ml). After 10 minutes, the clear reaction solution was diluted with ethyl acetate (40 ml) and washed with water (4 × 20 ml), brine, and dried. Removal of the solvent *in vacuo* gave a gum which was purified by flash chromatography on silica gel (15 g) using a 75% solution of ethyl acetate in cyclohexane as eluant to give the title compound.

NMR: δ = 1.44 (3H, s), 1.59 (3H, s), 2.18 (3H, s), 2.59 (1H, bs), 3.40 (2H, ABq, *J* = 19 Hz), 4.15 (3H, m), 4.52 (1H, s), 4.72 (1H, d, *J* = 4.4 Hz), 5.00 (1H, d, *J* = 4.7 Hz), 5.13 (1H, s), 5.72 (1H, dd, *J* = 9 Hz and 4.7 Hz), 5.94 (2H, s), 7.18 (1H, d, *J* = 9 Hz), and 7.40 (5H, s).

EXAMPLE 6

6β-Hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 7β-(D-α-benzyloxycarbonylamino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate

N-Benzyloxycarbonyl-cephalexin (0.48 g) was suspended in a solution of sodium hydrogen carbonate (0.2 g) and tetrabutylammonium hydrogen sulphate (0.39 g) in water (20 ml) and the mixture shaken with dichloromethane (20 ml). When all the benzyloxycarbonyl-cephalexin had passed into solution, the pH of the aqueous phase was adjusted to 8.0 by the addition a little saturated sodium hydrogen carbonate solution, and, after shaking again, the dichloromethane phase was separated, dried, and concentrated *in vacuo* to approximately 2 ml. This solution of the tetrabutylammonium salt of *N*-benzyloxycarbonyl-cephalexin was treated with a solution of iodomethyl 6β-hydroxymethyl-1,1-dioxopenicillanate (0.40 g) in ethyl acetate (5 ml). After 15 minutes, the reaction mixture was partially concentrated *in vacuo* to remove the dichloromethane, diluted to 20 ml with ethyl acetate, and extracted with water (20 ml). The ethyl acetate layer was dried and concentrated to give a product which was purified by chromatography on silica gel (30 g) using a 75% solution of ethyl acetate in cyclohexane as eluant to give the title compound.

EXAMPLE 7

6β-Hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 7β-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate, hydrochloride

Hydrogen gas was bubbled through a well-stirred mixture of a solution of 6β-hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 7β-(D-α-azido-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate (100 mg) in ethyl acetate (20 ml), water (20 ml), and 10% palladium on active carbon (100 mg), while the pH of the mixture was maintained between 2.4 and 2.6 by the addition of N/10 hydrochloric acid dropwise over 40 minutes. The reaction mixture was filtered through filter aid, and the aqueous phase was separated and freeze-dried to give the title compound as a solid.

NMR: δ = 1.35 (3H, s), 1.49 (3H, s), 2.04 (3H, s), 3.3 (2H, ABq, *J* = 18 Hz), 4.1 (3H, m), 5.17 (1H, s), 5.56 (1H, d, *J* = 4.4 Hz), 5.93 (2H, s), and 7.45 (5H, s).

The title compound could also be prepared by substituting 6β-hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 7β-(D-α-benzyloxycarbonylamino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate (100 mg) for the above starting material, but otherwise the reaction procedure remains unchanged.

The title compound could be further be produced by suspending cephalexin (0.35 g) in dichloromethane (10 ml) and adding dropwise 1.5 M tetrabutylammonium hydroxide aqueous solution (0.67 ml). The clear, two-phase mixture thus obtained was concentrated to constant weight *in vacuo* to give cephalexin tetrabutyl ammonium salt. This was redissolved in dichloromethane (2 ml) and treated with a solution of iodomethyl 6β-hydroxymethyl-1,1-dioxopenicillanate (0.40 g) in ethyl acetate (5 ml). After 15 minutes the reaction mixture was partially concentrated *in vacuo* to remove the dichloromethane, diluted to 20 ml with ethyl acetate, and washed with water (2 × 10 ml). The ethyl acetate layer was then extracted with water (20 ml) at pH 2.5 (maintained by the addition of the necessary amount of 1N hydrochloric acid). The aqueous extract was separated and freeze-dried to give the title compound as a solid.

EXAMPLE 8

6β-Hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 7β-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate

A sample (20 mg) of the hydrochloride from Example 7 was dissolved in water (5 ml), ethyl acetate (10 ml) was added, and the stirred mixture was treated with saturated sodium hydrogen carbonate solution (1 ml).

After separation, the ethyl acetate layer was dried and concentrated *in vacuo* to give the title compound. NMR: δ = 1.45 (3H, s), 1.59 (3H, s), 2.16 (3H, s), 3.39 (2H, ABq, *J* = 18 Hz), 4.2 (3H, m), 4.52 (1H, s), 4.59 (1H, s), 472 (1H, d, *J* = 4.1 Hz), 4.99 (1H, d, *J* = 4.7 Hz), 5.75 (1H, dd, J = 10 Hz and 4.7 Hz), 5.94 (2H, s), 7.36 (5H, s), and 7.91 (1H, d, J = 10 Hz).

EXAMPLE 9

Following the procedure of Example 7, last method, but by substituting 1 mmol of the appropriate cephalosporin for the cephalexin, the hydrochlorides of the 6β-hydroxymethyl-1,1-dioxopenicillanoyloxymethyl esters of the following compounds were prepared:
a) cefachlor, b) cefadroxil, c) cefatrizine, d) cephradine, and e) cefroxadine.

EXAMPLE 10

6β-Hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 6β-[(hexahydro-1H-azepin-1-yl)methyleneamino]penicillanate

Sodium iodide (2 g) was added to a stirred solution of chloromethyl 6β-[(hexahydro-1*H*-azepin-1-yl)methyleneamino]penicillanate (mecillinam chloromethyl ester) (0.5 g) in acetone (5 ml), and stirring was continued at room temperature for 3 hours. The reaction mixture was partitioned between ethyl acetate (30 ml) and water (30 ml), and the ethyl acetate layer was separated, washed with 5% sodium hydrogen carbonate solution containing a few drops of 10% sodium thiosulphate solution, brine, and dried. Removal of the solvent *in vacuo* gave a gum. This was taken up in dry *N,N*-dimethylformamide (DMF) (5 ml) and an aliquot (2.0 ml) of the solution was added to a stirred suspension of potassium 6β-hydroxymethylpenicillanate 1,1-dioxide (75 mg) in dry DMF (1 ml). After stirring for 10 minutes the reaction mixture was partitioned between water (25 ml) and ethyl acetate (35 ml). The ethyl acetate layer was washed with water (3 × 20 ml), brine, and dried. Removal of the solvent *in vacuo* gave a gum which was purified by colomn chromatography on Sephadex® LH 20 using a 65% solution of chloroform in hexane as eluant to give the title compound.
NMR: δ = 1.58 (6H, s), 1.67 (6H, s), 1.5 (8H, m), 3.09 (1H, bs), 3.35 (4H, m), 4.2 (3H, m), 4.39 (1H, s), 4.49 (1H, s), 4.71 (1H, d, *J* = 4 Hz), 5.10 (1H, d, J = 4 Hz), 5.45 (1H, d, J = 4 Hz), 5.89 (2H, ABq, *J* = 6 Hz), and 7.61 (1H, s).

EXAMPLE 11

6β-Hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 6β-[(hexahydro-1H-azepin-1-yl)methyleneamino]penicillanate

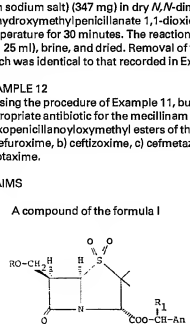
To a stirred suspension of sodium 6β-[(hexahydro-1*H*-azepin-1-yl)methyleneamino]penicillanate (mecillinam sodium salt) (347 mg) in dry *N,N*-dimethylformamide (DMF) (5 ml) was added a solution of iodomethyl 6β-hydroxymethylpenicillanate 1,1-dioxide (403 mg) in dry DMF (5 ml) and stirring was continued at room temperature for 30 minutes. The reaction mixture was diluted with ethyl acetate (70 ml), washed with water (4 × 25 ml), brine, and dried. Removal of the solvent *in vacuo* gave the title product, the NMR spectrum of which was identical to that recorded in Example 10.

EXAMPLE 12

Using the procedure of Example 11, but substituting 1 mmol of the sodium or potassium salt of the appropriate antibiotic for the mecillinam sodium salt, the 6β-hydroxymethyl-1,1-dioxopenicillanoyloxymethyl esters of the following antibiotics were prepared:
a) cefuroxime, b) ceftizoxime, c) cefmetazole, d) 3'-desacetoxy-cefotaxime, e) 3'-methoxy-3'-desacetoxy-cefotaxime.

CLAIMS

1. A compound of the formula I

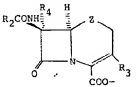


I

in which R = H, C_1-C_4 alkyl or C_2-C_6 acyl, R_1 stands for hydrogen, methyl, ethyl benzyl, or phenyl, and An stands for a radical of a cephalosporin-type or an amidinopenicillanic acid being connected via the carboxy group; and pharmaceutically acceptable, non-toxic salts of the compound of formula I.

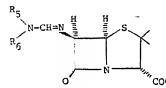
2. The pure diastereomers of the formula I of claim 1, as well as salts of the diastereomers and mixtures thereof, in case R and/or the ester moiety contain a chiral center.

3. A compound of formula I of claim 1, in which An is a radical selected from the group consisting of



IIa, Z = S

IIb, Z = O



III

in which formulae R_2CONH- stands for the 7-side chain of a known cephalosporin, R_3 stands for the 3-substituent of a known cephalosporin, R_4 stands for hydrogen or methoxy, and

stands for the side chain of a known amidinopenicillanic acid; and pharmaceutically acceptable, non-toxic salts thereof.

4. A compound according to claim 3, in which An stands for a radical of formula IIa, R_2 stands for D-α-aminobenzyl, D-α-amino-*p*-hydroxybenzyl, or D-α-amino-α-(1,4-cyclohexadien-1yl)methyl, R^3 stands for chlorine, methyl, methoxy, or 1,2,3-triazol-5-ylthiomethyl, R_4 stands for hydrogen.

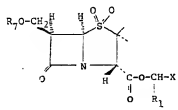
5. A compound according to claim 3, in which An stands for a radical of formula III, and R_5 and R_6 together with the nitrogen to which they are attached form a heterocyclic ring.

6. 6β-Hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 7β-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate and pharmaceutically acceptable, non-toxic salts thereof.

7. 6β-Hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 6β-[(hexahydro-1*H*-azepin-1-yl)methyleneamino]penicillanate and pharmaceutically acceptable, non-toxic salts thereof.

8. A method for producing a compound of claim 1, in which

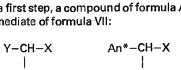
a) a compound of formula IV:



IV

in which R_1 is as defined before, R_7 is optionally R (as defined above) or an easily cleavable esterifying or etherifying group, and X stands for a leaving group, is reacted with a compound of formula $An^*{}^{\oplus}M^{\oplus}$, in which An* is optionally An (as defined above) or a protected or masked derivative of An, and $M^{\oplus}$ is a cation, followed, when $R_7 \neq An$, by appropriate chemical modifications to replace R_7 with hydrogen and then optionally alkylation or acylation, and/or reactions required to convert An* to An, to give the desired compound of formula I; or

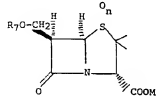
b) as a first step, a compound of formula $An^*{}^{\oplus}M^{\oplus}$ is reacted with a compound of formula VI to afford an intermediate of formula VII:



VI VII

in which R_1, A*, and X are as defined before, and Y represents a leaving group, Y being a better leaving

group than X, whereafter in a second step the intermediate of formula VII, optionally after modification of An* and/or substitution of X for a better leaving group, is reacted with a compound of the formula Va



Va, n = 2
Vb, n = 0

in which R_7 and M have the above meanings, to form the desired compound of formula I, if necessary, after appropriate modification of R_7 to R and/or An* to An.

9. Method according to claim 8 b), in which the compound of formula VI is chloromethyl chlorosulphate, and the reaction is performed in a two-phase solvent system including water with a phase transfer catalyst, in the presence of a base.

10. A pharmaceutical preparation in dosage unit form for enteral, parenteral or topical treatment of patients (including animals) suffering from infectious diseases, which comprises as an active ingredient 0.050 g to 2.5 g of a compound as claimed in claim 1 together with an atoxic pharmaceutically acceptable carrier.

11. A pharmaceutical preparation in dosage unit form as claimed in claim 10 for oral treatment of patients, containing from 0.1 g to 1 g of the active ingredient.

12. A pharmaceutical preparation in dosage unit form as claimed in claim 10 and containing as the active component the compound 6β-hydroxymethyl-1-1,dioxopenicillanoyloxymethyl 6β-[(hexahydro-1H-azepin-1-yl)-methyleneamino]-penicillanate or a salt thereof with a pharmaceutically acceptable, non-toxic acid.

13. A pharmaceutical preparation in dosage unit form as claimed in claim 10 and containing as the active component the compound 6β-hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 7β-(D-α-amino-α-phenylacetamido)-3-methyl-3-cephem-4-carboxylate or a salt thereof with a pharmaceutically acceptable, non-toxic acid.

14. A pharmaceutical preparation in dosage unit form as claimed in claim 12-13 in the form of tablets, pills, or capsules.

15. A pharmaceutical composition containing a compound as claimed in claim 1 together with carrier substances and auxiliary agents, containing from 1% to 95% of the active compound.

16. A compounded pharmaceutical composition as claimed in claim 15 containing the active ingredient together with a known penicillin, the ratio between the active compounds being between 1:20 and 20:1, preferably between 1:5 and 5:1.

17. A compounded pharmaceutical composition as claimed in claim 16, in which the active ingredient is 6β-hydroxymethyl-1,1-dioxopenicillanoyloxymethyl 6β-[(hexahydro-1H-azepin-1-yl)-methyleneamino]penicillanate, optionally being used in the form of a salt with a pharmaceutically acceptable, non-toxic acid, and the pencicillin is 6-[D-α-amino-α-phenylacetamido]penicillanic acid.

18. A compound of the formula I defined in Claim 1 substantially as hereinbefore described in any one of the foregoing Examples.

19. A method of producing a compound of the formula I defined in Claim 1 substantially as hereinbefore described in any one of the foregoing Examples.

20. A pharmaceutical preparation in dosage unit form in accordance with Claim 10 substantially as hereinbefore described.

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